November 18, 2005

Mr. Mike Gallagher
PBT Coordinator
Dept. of Ecology, Washington State
PO Box 47600
Olympia, WA 98504
e-mail: mgal461@ecy.wa.gov

Dear Mr. Gallagher:

The Alkylphenols & Ethoxylates Research Council (APERC)[1] submits the
enclosed comments on the Department of Ecology's Revised Draft Washington State
Rule, Chapter 173-333 WAC on Persistent Bioaccumulative Toxins (PBTs) (September
29, 2005), which proposes to list nonylphenol and 4-nonylphenol, branched (CAS
numbers 25154-52-3 and 84852-15-3) to Washington State's PBT list. APERC is
comprised of major North American producers and users of alkylphenols (AP) and
alkylphenol ethoxylates (APE) including nonylphenol (NP), and nonylphenol ethoxylates
(NPE). The Council has been actively engaged in environmental fate and effects research
on NP for over 20 years and consequently can contribute considerable information and
expertise relevant to Washington State Department of Ecology's PBT assessment of these
substances.

The attached comments support the conclusions that NP is biodegradable, is not
persistent, is not bioaccumulative and therefore should not be categorized as a PBT. In
addition, the comments address APERC's concern with the following issues in the
Revised Draft Rule on PBTs:

**Washington State Department of Ecology (Ecology) should conduct thorough
assessments that consider the highest quality data available utilizing a scientifically-
based approach <u>before</u> listing compounds on a proposed PBT list.**

The market implications for a compound proposed as a PBT by any regulatory authority
are grave even if a compound is subsequently removed from the list. A PBT designation
generally infers a need to essentially eliminate the use of a compound. Ecology's
intention that its definition of PBT "does not represent a decision that all uses and
releases of that chemical should be reduced and phased-out" will not necessarily be
recognized by other regulatory authorities or the businesses and consumers that use

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[1] Members of the Alkylphenols & Ethoxylates Research Council include: Dover Chemical Corporation; Rhodia Inc.;
Rohm and Haas Company; Schenectady International, Inc.; and, The Dow Chemical Company.

products containing compounds incorrectly designated as PBTs. Considering the likelihood that Ecology's intention regarding a PBT listing could be misperceived and result in unnecessary market disruption and economic impact to affected businesses, Ecology has an obligation to conduct thorough and scientifically-based assessments prior to listing any compound as PBT.

Ecology should conduct assessments that are consistent with those generally accepted in the scientific community; utilizing a preferred hierarchy of data sources where the weight-of-evidence is preferred over single measured values, which are in turn preferred over estimated, calculated or modeled values.

The Revised Draft PBT Rule states that listings will be based on "credible scientific information," which is defined as "information that is based on a theory or technique that is generally acceptable in the relevant scientific community or has been collected or derived using standard or generally accepted methods and protocols and appropriate quality assurance and control procedures." Ecology should adopt a policy on hierarchy of data sources that is consistent with other regulatory authorities and the scientific community in general.

The evaluation that Ecology provided as support for listing NP as a PBT chemical is inadequate in terms of effort, completeness and scientific rigor.

Ecology provides a superficial three paragraph review of persistence, bioaccumulation and toxicity data for NP, which is one of the most extensively tested chemicals in commerce today. Literally hundreds of studies have been conducted and are readily available in the peer-reviewed published literature as well as in other publicly available sources. The fact that the sheer volume of available data on a compound may seem overwhelming does not provide Ecology with an excuse to disregard it; rather it obligates a weight-of-evidence assessment.

The values selected by Ecology to justify listing NP as either persistent or bioaccumulative are from inadequate quality data sources, especially in light of the fact that there are numerous other high quality data available from which to conduct a weight-of-evidence assessment.

Perhaps the most disturbing aspect related to the listing of NP on the proposed Washington State PBT list in the Revised Draft Rule (September 29, 2005) was the fact that Ecology acknowledges the extensive comments and scientific references provided by APERC in 2001 while apparently choosing to ignore the data - already in hand - that support with well-founded science the conclusion that NP does not meet the proposed Washington State criteria for persistence and bioaccumulation. Ecology should use the extensive body of high quality data, which are summarized in the attached comments, to conduct its assessment on NP. These data support a weight-of-evidence based conclusion that NP should not be classified as PBT.

The fact that the attached APERC comments do not address Ecology's toxicity assessment on NP should not be interpreted to indicate APERC's agreement with that assessment. Clearly Ecology's one paragraph summary of the toxicity of NP is not adequate to address the extensive toxicity data available for this compound.

As noted in Ecology's Technical Background Information document, US EPA proposed Water Quality Criteria (WQC) for NP in January 2004 that are protective of acute and chronic effects in fresh and salt water organisms.[2] Toxicity alone does not justify listing any compound as a PBT. As with any other chemical, WQC form the basis for responsible chemical management. The results of a statistical analysis of environmental exposures and a probabilistic risk assessment of the impact of NP on aquatic ecosystems in the United States support the understanding that there is only a low probability that levels of NP in the aquatic environment exceed US EPA's proposed WQC for NP and that this compound is being effectively managed in this country.[3]

The American Chemistry Council and the Association of Washington Business have also submitted comments related to the Department of Ecology's proposed PBT strategy. APERC also supports these more general comments, especially as they relate to the definition of PBT criteria and the need for coordination with federal and international initiatives.

If you have any questions or wish to discuss these comments in more detail please contact me at (202) 419-1500.

Sincerely,

Barbara S. Losey
Deputy Director

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[2] US EPA. (2003). Ambient Aquatic Life Water Quality Criteria for Nonylphenol - Draft. EPA 822-R-03-029. http://www.epa.gov/waterscience/criteria/nonylphenol/draft-nonylphenol.pdf

[3] Zabik, J.M., Klecka, G.M., Woodburn, K.B., Naylor, C.G., and Staples, C.A. (2005). Exposure Analysis of Alkylphenol, Alkylphenol Ethoxylates and their Metabolites in US Surface Waters. Poster Presentation Abstract Number KLE-1117-827542. Society of Environmental Toxicology and Chemistry (SETAC) 26th Annual Conference, Baltimore, MD.

ALKYLPHENOLS & ETHOXYLATES RESEARCH COUNCIL
COMMENTS ON
WASHINGTON STATE REVISED VERSION OF
PROPOSED PBT RULE, CHAPTER 173-333
(SEPTEMBER 29, 2005)

November 18, 2005

The Alkylphenols & Ethoxylates Research Council (APERC) is pleased to submit these comments on the Department of Ecology's Revised Draft Washington State Rule, Chapter 173-333 WAC on Persistent Bioaccumulative Toxins (PBT) (September 29, 2005),[1] which proposes to add nonylphenol/4-nonylphenol, branched (CAS numbers 25154-52-3 and 84852-15-3) to the proposed PBT list. APERC is comprised of major North American producers and users of alkylphenols (AP) and alkylphenol ethoxylates (APE) including nonylphenol (NP), and nonylphenol ethoxylates (NPE).[2] For more than twenty years APERC, its predecessor the American Chemistry Council's Alkylphenols and Ethoxylates Panel, and its member companies have been actively engaged in environmental fate and effects research on APs and APEs. Consequently, APERC can contribute considerable information and expertise relevant to the environmental assessment of these substances.

As noted in Appendix B of the Revised Draft PBT Rule, APERC provided extensive comments and scientific references to the Department of Ecology in 2001 that support with well-founded science the conclusion that NP is neither persistent nor bioaccumulative and should not be identified on Washington State's candidate PBT list.[3] Unfortunately, much of the data provided in those comments has been ignored in Ecology's brief evaluation of "readily available" information on the persistence and bioaccumulation potential of NP.[4]

In addition to presenting APERC's concerns regarding the superficial PBT assessment on NP conducted by Ecology, these comments explain why the persistence (P) and bioaccumulation (B) values selected by Ecology are inappropriate to justify listing NP as a PBT chemical considering the availability of other more appropriate values. These comments also provide a technical review of readily available studies on the persistence and bioaccumulation potential of NP that will allow a more comprehensive assessment that supports the conclusion that NP is not a PBT chemical.

I.   APERC CONCERNS WITH ECOLOGY'S PBT ASSESSMENT OF NP

The Revised Draft PBT Rule indicates that the Department of Ecology will base decisions about PBT chemicals "on sound public policy and *credible scientific information*" and defines the latter to mean "information that is based on a theory or technique that is generally acceptable in the relevant scientific community or has been collected or derived using standard or generally accepted methods and protocols and appropriate quality assurance and control procedures."[5] Therefore, it is surprising that the PBT evaluation of NP presented in the Summary Technical Background Information

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[1] Washington State Department of Ecology. (2005, September 29). Proposed PBT Rule. http://www.ecy.wa.gov/laws-rules/wac173333/p0407_cont_a.pdf
[2] Members of the Alkylphenols & Ethoxylates Research Council include: Dover Chemical Corporation; Rhodia Inc.; Rohm and Haas Company; Schenectady International, Inc.; and, The Dow Chemical Company.
[3] Alkylphenols & Ethoxylates Research Council. (2000, December). Comments on the Proposed Strategy To Continually Reduce Persistent Bioaccumulative Toxins (PBTs) in Washington State.
[4] Washington State Department of Ecology. (2005, October). Summary Technical Background Information for the Proposed PBT List: Revised Draft. http://www.ecy.wa.gov/programs/eap/pbt/rule/docs/Summary-TechnicalBackgroundInformationforProposedPBTList(October2005-Bradley).doc
[5] Washington State Department of Ecology. (2005, September 29). Proposed PBT Rule. http://www.ecy.wa.gov/laws-rules/wac173333/p0407_cont_a.pdf

document for the Revised Draft PBT Rule is so brief, superficial and ignores extensive readily available data as well as the technical comments with numerous scientific references submitted to Ecology by APERC in 2001. Considering that the market implications for any compound classified as a "PBT" by any regulatory authority are grave, Ecology should conduct extremely thorough assessments that consider all available data, utilize a scientifically-based approach and give preference to the highest quality data available. While the Revised Draft PBT Rule provides a process for removing a PBT chemical from Washington State's list, the commercial damage caused by an incorrect classification and listing of a compound can be devastating even if the compound is subsequently removed. As such, Ecology should conduct thorough, science-based assessments prior to listing a compound as a PBT - even on a proposed list.

The review of "readily available" data on NP listed in the Summary Technical Background Information document appears to have been based on a minimal effort to review several assessment documents and models to identify a few P and B values that exceed the proposed criteria. This approach is not consistent with the approach generally accepted in the scientific community that utilizes a preferred hierarchy of data sources in the assessment of chemicals; more specifically, consideration of the weight of the evidence is preferred over single measured values, which are in turn preferred over estimated, calculated or modeled values.

The process of determining the quality of existing data is well established and takes into consideration three aspects - reliability, relevance and adequacy of the data. These terms as defined by Klimisch et al. (1997)[6] are generally accepted in the scientific community as well by governmental authorities as follows.

- Reliability: The inherent quality of a test report or publication relating preferably to standardized methodology and the way the experimental procedure and results are described to give evidence of the clarity and plausibility of the findings. Reliability addresses the overall scientific integrity and validity of the information in a study;
- Relevance: The extent to which data and tests are appropriate for a particular hazard identification or risk characteristic; and,
- Adequacy: The usefulness of data for hazard/risk assessment purposes.

Governmental authorities such as the US Environmental Protection Agency (EPA) and the Organization for Economic Cooperation and Development (OECD) routinely require characterization of data quality in risk assessments and data submission such as those conducted under the High Production Volume (HPV) Challenge Program.[7] In order to be consistent with the hierarchy of data sources approach generally accepted in the scientific and regulatory communities, Washington State should assign the greatest weight to studies that are the most reliable, relevant and adequate. Studies that do not meet these criteria should be considered only as supplementary information.

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[6] Klimisch, H.J., Andreae, E., and Tillmann, U. (1997). A Systematic Approach for Evaluating the Quality of Experimental and Ecotoxicological Data. Reg. Tox. and Pharm., 25,1-5.
[7] US EPA. (2004). High Production Volume Challenge Program. Guidance for Meeting SIDS Requirements. http://www.epa.gov/opptintr/chemrtk/sidsappb.htm

Most of the pivotal P and B values listed in Ecology's PBT assessment of NP are based on estimated, modeled or calculated values. The others are based on measured values, which upon detailed examination do not meet the data quality criteria to be classified as "reliable without restrictions." Certainly, if there are inadequate data available one might turn to lesser quality data for screening purposes; however, this is not the case for NP. An extensive data set exists on the P, B and environmental fate characteristics of NP, which has been summarized in readily available review articles.[8,9] In addition, various regulatory authorities have published risk assessments on NP and none have resulted in its categorization as a PBT compound according to international standards.[10,11,12] Finally, numerous other high quality studies on the P and B characteristics of NP are readily available in the published literature and should be considered with greater weight in Ecology's assessment of this compound.

Furthermore, an evaluation based solely on the PBT characteristics of a compound versus a set of criteria does not represent the risk presented by the chemical. The process for developing, amending and removing chemicals from the PBT list should include an assessment of exposure levels in Washington State to determine whether the compound does in fact represent a risk. As noted in the Technical Background Information document, US EPA proposed Water Quality Criteria (WQC) for NP in January 2004 that are protective of acute and chronic effects in fresh and salt water organisms.[13] The results of a statistical analysis of environmental exposures and a probabilistic risk assessment, conducted by APERC, for NP impact on aquatic ecosystems in the US, support the understanding that there is only a low probability that levels of NP in the aquatic environment exceed US EPA's proposed WQC for NP.[14] As discussed below, NP is biodegradable, is not persistent, is not bioaccumulative and is being effectively managed in this country.

II.   DESCRIPTION OF SUBSTANCE

Nonylphenol is the commercial description for a complex mixture of nine-carbon alkyl-chain substituted phenols. Several CAS numbers are available for NP, though CAS numbers 25154-52-3 (nonylphenol) and 84852-15-3 (4-nonylphenol, branched), which are identified in the Revised Draft PBT Rule, are the most commercially relevant.

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[8] Staples, C.A., Weeks, J., Hall, J.F., and Naylor, C.G. (1998). Evaluation of aquatic toxicity and bioaccumulation of C8- and C9-alkylphenol ethoxylates. Environ. Toxicol. Chem., 17(12), 2470-2480.

[9] Servos, M.R. (1999). Review of the aquatic toxicity, estrogenic responses and bioaccumulation of alkylphenols and alkylphenol polyethoxylates. Water Qual. Res. J. Canada, 34, 123-177.

[10] Environment Canada and Health Canada. (2001). Priority Substances List Assessment Report: Nonylphenol and Its Ethoxylates.

[11] European Union. (2001). Risk Assessment Report: 4-Nonylphenol (branched) and Nonylphenol: Final Report. http://ecb.jrc.it/DOCUMENTS/Existing-Chemicals/RISK_ASSESSMENT/REPORT/

[12] European Chemicals Bureau. (2003). PBT Working Group Substance Information Sheets for Nonylphenol (CAS 25154-52-3) and Phenol, 4-Nonyl, branched (CAS 84852-15-3).

[13] US EPA. (2003). Ambient Aquatic Life Water Quality Criteria for Nonylphenol - Draft. EPA 822-R-03-029. http://www.epa.gov/waterscience/criteria/nonylphenol/draft-nonylphenol.pdf

[14] Zabik, J.M., Klecka, G.M., Woodburn, K.B., Naylor, C.G., and Staples, C.A. (2005). Exposure Analysis of Alkylphenol, Alkylphenol Ethoxylates and their Metabolites in US Surface Waters. Poster Presentation Abstract Number KLE-1117-827542. Society of Environmental Toxicology and Chemistry (SETAC) 26th Annual Conference, Baltimore, MD.

NP is produced through the Friedel-Crafts alkylation of phenol with nonene in the presence of an acid catalyst, which preferentially alkylates at the *para* position of phenol. Commercial nonene does not contain linear C_9H_{18} *alpha*-olefin; rather it is a complex mixture of highly branched, predominantly nine-carbon olefins known as propylene trimers. Therefore, NP formed by the alkylation of phenol with propylene trimers is also a very complex mixture of branched isomers with the following approximate composition: *ortho*-NP (3-6%), *para*-NP (90-93%) and decylphenol (2-5%). Since the *para* isomer predominates, the product is most accurately described as *para*-nonylphenol, branched (*p*-NP or PNP).[15] The following table lists the results of high-resolution gas chromatography analyses of *p*-NP and identifies 22 branched *para*-isomers, within five distinct groups. Group designations are presented based on the substitution of the *alpha*-carbon on the alkyl chain.[16]

Table 1. Para Isomers of Nonylphenol

Group #	Isomer Type	Number of Isomers	Para Isomers %
1	Alpha-dimethyl	10	48.6%
2	Alpha-methyl, alpha- ethyl, beta-primary	3	8.9%
3	Alpha-methyl, beta-methyl	4	24.7%
4	Alpha-methyl	2	6.6%
5	Alpha-methyl, alpha- propyl	3	11.2%
	Total Isomers	22	100%

The complexity of the isomers represented in commercial NP has been recognized in US EPA's proposed WQC and Risk Management 1 Documents for NP as well as in Environment/Health Canada's Priority Substance List Assessment for NP and NPE and the European Union (EU) Risk Assessment on NP and its ethoxylates.[17,18,19,20] Following is a table that summarizes CAS numbers for NP, their nomenclature and related governmental assessments.

Table 2. Nonylphenol Descriptions, Structures and Governmental Assessments

CAS Number	Description	Governmental Assessments	Structure (Sources: CAS, NIST, APERC)
84852-15-3	Phenol, 4-nonyl-, branched **Other** Nonylphenol; 4-Nonylphenol; p-Nonylphenol,	US EPA (1996) EC CEPA (2000) EU (2001) ECB (2003) US EPA (2003)	Unspecified, mixed isomers

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[15] Bhatt, B.D., Prasad, J.V., and Ali, S. (1992). Separation and characterization of isomers of *p*-nonylphenols by capillary GC/GC-MS/GC-FTIR techniques. J. Chromatographic Sci., 30, 203-210.

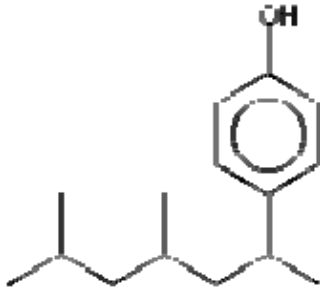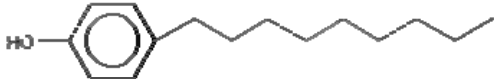[16] Wheeler, T.F., Heim, J.R., LaTorre, M.R., and Janes, A.B. (1997). Mass Spectral Characterization of *p*-Nonylphenol Isomers using High-Resolution Capillary GC-MS. Journal of Chromatographic Science, 35(1).

[17] Environment Canada and Health Canada. (2001). Priority Substances List Assessment Report: Nonylphenol and Its Ethoxylates.

[18] US EPA. (2003). Ambient Aquatic Life Water Quality Criteria for Nonylphenol - Draft. EPA 822-R-03-029. http://www.epa.gov/waterscience/criteria/nonylphenol/draft-nonylphenol.pdf

[19] European Union. (2001). Risk Assessment Report: 4-Nonylphenol (branched) and Nonylphenol: Final Report. http://ecb.jrc.it/DOCUMENTS/Existing-Chemicals/RISK_ASSESSMENT/REPORT/.

[20] Rodier, D. (1996). EPA RM-1 Document for Para-Nonylphenol.

	branched; Branched 4-nonylphenol (mixed isomers)		
25154-52-3 **Other** 84852-15-3 **Former** 1300-16-9; 56459-00-4	Phenol, nonyl **Other** Phenol, nonyl-; x-Nonylphenol; Nonylphenol (mixed isomers); Nonylphenol (mixture)	US EPA (1996) EC CEPA (2000) EU (2001) ECB (2003) US EPA (2003)	
104-40-5	p-Nonylphenol **Other** 4-Nonylphenol; Phenol, p-nonyl-; 4-n-Nonyl phenol; Phenol, 4-n-nonyl	US EPA (1996)	

In summary, based on APERC's understanding there are in principle two forms of NP- linear and branched. Linear NP is described by CAS number 104-40-5; however it is not available other than in small research quantities. Commercially relevant NP is a mixture of isomers with various levels of branching and is best described by CAS number 84852-15-3, though CAS number 25154-52-3 is also used. While the use of models (e.g., EPA PBT Profiler,[21] WMPT model[22]) to predict P and B properties of compounds is always less reliable than experimental data and actual measured half-lives, the complexity of the mixture of isomers in commercially available NP makes results from model estimations even less reliable.

III. PERSISTENCE

Department of Ecology Review of Persistence

Ecology's review of "readily available" persistence information on NP consists of the following short paragraph.

> *UNEP (2002) concluded that "...NPs and t-OP are persistent in the environment with half-lives of 30-60 years in marine sediments, 1-3 weeks in estuarine waters and 10-48 hours in the atmosphere..." The European Union (ECB, 2002) reviewed the results of several biodegradation tests and estimated biodegradation half lives of 150 days and 300 days in surface water and soils, respectively. The*

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[21] US EPA. (2004b). PBT Profiler. Prepared by Environmental Science Center for the Office of Pollution Prevention and Toxic Substances, US EPA. Version 1.203.
[22] US EPA. (1998, September). Waste Minimization Prioritization Tool Spreadsheet Document for the RCRA Waste Minimization PBT Chemical List Docket (# F-98-MMLP-FFFFF).

media- specific half-life values predicted by the EPA PBT Profiler (EPA, 2004) are surface water (15 days), soil (30 days) and sediments (140 days).[23]

Ecology cites a marine sediment half-life of 30 -60 years in the United Nations Environment Programme (UNEP) North American Regionally Based Assessment of Persistent Toxic Substances.[24] The primary reference for this half-life range is a single sediment study by Shang (1999)[25] that was conducted on NP buried in deep, anoxic marine sediment, an environmental compartment that is not relevant to risk assessments or persistence definitions. Even the authors suggest that the NP in the sediment is entrained and therefore not biologically relevant noting, "If preservation is accomplished, as we suggest, by irreversibly sequestering into organic coatings on solids, NPnEOs may not be bioavailable." Furthermore, mass balance models,[26] which link emission rates to prevailing environmental concentrations include consideration of chemical degradation, partitioning and transport among all relevant media, consider burial to deep inaccessible and usually anaerobic sediments as a permanent loss from the biosphere. Since the surface layers of aquatic sediments are aerobic and contain higher levels of microorganisms, degradation of NP occurs in this more relevant sediment compartment. Therefore, should anaerobic sediments become re-suspended, any adsorbed NP would be expected to resume degradation under aerobic conditions.

Also, the 30 to 60 year marine sediment half-life range and the Shang (1999) citation were removed in the UNEP Regionally Based Assessment of Persistent Toxic Substances - 2nd Draft Global Report,[27] which is a compilation of all the UNEP regional reports that considered comments submitted from stakeholders. Since the Shang (1999) study does not represent persistence in a bioavailable compartment, it does not meet the data quality criteria for relevance and adequacy; therefore, Ecology should not include the 30-60 year marine sediment half-life in its assessment of the persistence of NP.

Ecology also cites a surface water half-life of 150 days and a soil half-life of 300 days in its paragraph on persistence. These values were estimated by the European Chemicals Bureau (ECB) based on general assumptions and conservative extrapolations of data for inherently biodegradable substances provided in the EU Technical Guidance document.[28,29] In light of the extensive available database of valid laboratory and field biodegradation half-life measurements on NP, the complexity of isomers in commercial NP and the generalized assumptions that led to these ECB estimates, these values should not be cited by Ecology as the basis for a persistent classification. For similar reasons, a

[23] Washington State Department of Ecology. (2005, September 29). Proposed PBT Rule. Attachment B, Discussion of NP Public Comments, September 5, 2005. http://www.ecy.wa.gov/laws-rules/wac173333/p0407_cont_a.pdf

[24] UNEP. (2002, December). North American Regionally Based Assessment of Persistent Toxic Substances.

[25] Shang, D.Y., Macdonald, R.W., and Ikonomou, M.G. (1999). Persistence of nonylphenol ethoxylate surfactants and their primary degradation products in sediments from a municipal outfall in the Strait of Georgia, British Columbia, Canada. Environ Sci Technol., 33, 1366-72.

[26] The ChemCAN model is widely being used in Canada. The BETR model applies to the entire continent of North America including Canada, US and Mexico. The Caltox model is widely used in California.

[27] UNEP. (2003). Regionally Based Assessment of Persistent Toxic Substances - 2nd Draft Global Report.

[28] ECB. (2002). European Union Risk Assessment Report. 4-nonylphenol (branched) and nonylphenol. Publication. EUR 20387 EN.

[29] European Chemicals Bureau. (2003). Technical Guidance Document. http://ecb.jrc.it/

predicted 140 day sediment half-life value based on model predictions derived using the EPA PBT Profiler[30] should not be cited for persistence categorization. As noted previously, valid experimental data should always take precedence over model calculations as the basis for the decision.

In summary, none of the half-life values cited in Ecology's short discussion of persistence of NP should be used as a basis for categorizing NP as persistent. Following is a discussion of the more relevant and extensive biodegradation data available in the published literature, which should be used in this evaluation.

Persistence - Other Readily Available Data That Ecology Should Consider

The environmental persistence of NP has been extensively studied using a variety of test systems, ranging from screening tests (ready and inherent biodegradability) to simulation tests (river die-away, soil degradation). Biodegradation is the dominant mechanism responsible for removal of NP from aquatic and terrestrial environments. Hydrolysis is not expected to be important, which is consistent with the compound's chemical structure and lack of functional groups susceptible to hydrolytic attack.

NP is not expected to be present in air due to its extremely low vapor pressure at 25°C, which ranges from 0.005 Pa (measured)[31] to 0.3 Pa. (extrapolated from measured values).[32] Mackay (2001) cited a narrower range of vapor pressures of 0.055 to 0.17 Pa with three values of about 0.07 Pa. [33] Atmospheric photo-oxidation half-lives for NP were calculated using EPISuite v3.12[34] assuming the highly branched isomer structure. The calculated vapor phase half-life for NP was 5 hours. This value suggests that sunlight mediated degradation in the atmosphere might play a role in the removal of NP from the environment. Based on the results of multimedia modeling, the most relevant compartments for persistence assessment are water and soil.

Biodegradation

The biodegradation of NP in the environment is well understood and has been studied by researchers for over 40 years. There is extensive published scientific literature available that supports this understanding, ranging from screening tests, which assess the ready and inherent biodegradability according to standard guidelines (e.g., OECD, EPA, etc), to simulation tests for surface waters, soils and wastewater treatment systems, which are designed to examine biodegradation in the laboratory under conditions that closely simulate the actual fate in the environment. While the earlier studies focused largely on

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[30] US EPA. (2004b). PBT Profiler. Prepared by Environmental Science Center for the Office of Pollution Prevention and Toxic Substances, US EPA. Version 1.203.

[31] Romano, R.R. (1991). Current studies on nonylphenol – physical/chemical, biodegradation and aquatic effects. p. 233-239. *In* Proceedings of the Seminar on Nonylphenol Ethoxylates (NPE) and Nonylphenol (NP). Saltsjobaden, Sweden, Feb. 6-8, 1991, Ingvar Bingman, Publ., Stockholm. ISBN 91-620-3907-5

[32] European Union. (2001). Risk Assessment Report: 4-Nonylphenol (branched) and Nonylphenol: Final Report. http://ecb.jrc.it/DOCUMENTS/Existing-Chemicals/RISK_ASSESSMENT/REPORT/.

[33] Mackay, D. (2001, February). A review of the environmental fate of nonylphenol and related substances. Canadian Environmental Modeling Center, Trent University.

[34] US EPA. (2000). AOPWIN v1.66.

elimination of parent material, recent improvements in analytical methods, use of radiotracers, and refinement and standardization of test methods have improved our understanding of the ultimate biological fate of NP, including ring cleavage and mineralization, in the environment. Further, microorganisms with the ability to utilize NP as a carbon source for growth have been isolated and described.[35]

By far the most common path of entry of NP to the environment is through its formation as a minor anaerobic biodegradation intermediate of nonylphenol ethoxylates, which are surfactants used in industrial, institutional and consumer products that are typically disposed down-the-drain. The degradation pathways of NPE have been reviewed in Ahel et al. (1994), DiCorcia et al. (1998) and Maguire (1999)[36,37,38] and are illustrated in Figure 1. The mechanism of NPE degradation proceeds initially by the sequential removal of ethoxylate units. Studies have shown that only minor amounts of NP are formed from NPE under anaerobic conditions and that under aerobic conditions, the aromatic ring is opened and the intermediate metabolites are further degraded to carbon dioxide.[39]

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[35] Soares, A., Guieysse, B., Delgado, O., and Mattiasson, B. (2003). Aerobic biodegradation of nonylphenol by cold-adapted bacteria. Biotechnol. Letters, 25, 731-738.
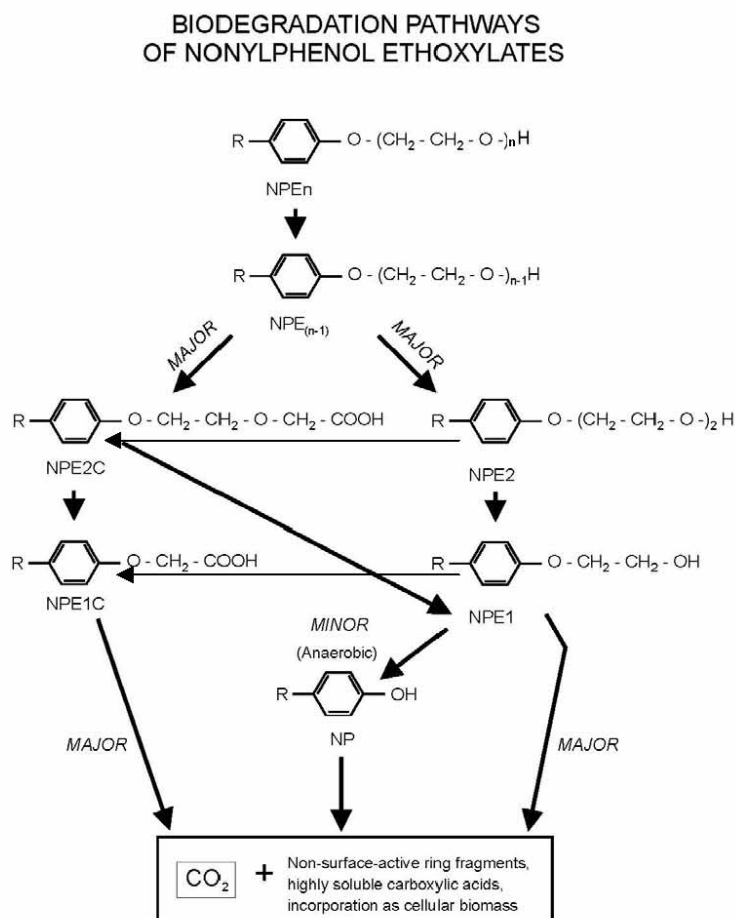
[36] Ahel, M., Giger, W., and Schaffner, C. (1994). Behavior of alkylphenol polyethoxylate surfactants in the aquatic environment – II. Occurrence and transformation in rivers. Water Research, 5, 1143-1152.

[37] DiCorcia, A., Costantino, A., Crescenzi, C., Marinoni, E., and Samperi, R. (1998). Characterization of recalcitrant intermediates from biotransformation of the branched alkyl side chain of nonylphenol ethoxylate surfactants. Environ. Sci. Technol., 32, 2401-2409.

[38] Maguire, R.J. (1999). Review of the persistence of nonylphenol and nonylphenol ethoxylates in aquatic environments. Water Qual. Research J. Canada, 34, 37-78.

[39] Naylor, C.G., Staples, C.A., Klecka, G.M., Williams, J.B., Varineau, P.T., and Cady, C. (2005). Biodegradation of [^{14}C] ring-labeled nonylphenol ethoxylate. Archives of Environmental Contamination and Toxicology. In-press.

Figure 1.

BIODEGRADATION PATHWAYS
OF NONYLPHENOL ETHOXYLATES

As will be discussed below, adequate information is publicly available to assess the environmental persistence of NP. The majority of studies, including both screening and simulation tests, have focused on commercially relevant NP and these values should take precedence over the calculated or predicted values cited by Ecology. Following is a summary of results of laboratory screening tests, simulation tests and field studies.

Ready Biodegradation Tests

Ready biodegradation tests are stringent methods that use relatively low concentrations of both microbial inoculum and substance concentration. The results of these tests are commonly used for screening or classification purposes. The test methods measure biodegradation by consumption of oxygen, removal of test substance (i.e., directly measure substance loss), removal of dissolved organic carbon, or by formation of carbon dioxide.

EPA provides guidance for using ready and inherent biodegradability data to environmental half-lives for use as input data for multimedia fate models,[40] which is summarized in Table 3 below.

Table 3. EPA Guidelines: Extrapolation of Ready and Inherent Biodegradation Test Results to Estimate Environmental Half-Lives

Ready Test Result Biodegradation at 28 days	Inherent Test Result	Water Half-life (days)
Passes	-	5
Does Not Pass; ≥40%	-	10
Does Not Pass; ≥20% and <40%	≥70%	30
	≥20% and ≤70%	100
Does Not Pass; <20%	<20%	10,000

This guidance will be used below to convert results of screening tests for NP for comparison with the Washington State persistence criteria. Staples et al. (1999, 2001) reported the results of ready biodegradation tests conducted according to Good Laboratory Practice on NP (CAS number 84852-15-3), which are summarized in Table 4 below. Based on CO_2 production observed in the OECD 301B test NP degraded by 48%. Results of the OECD 301F test also confirmed that NP was extensively degraded, with 62% of the theoretical oxygen demand consumed after 28 days.

Table 4. Summary of Extrapolation of Ready Biodegradability to Half-Lives for NP

Compound	CAS Number	Test Method	Results	Estimated Aquatic Half-life (days)	Reference
NP	84852-15-3	OECD301B (GLP)	48.0% ThCO2	**10**	Staples et al., 1999, 2001
NP	84852-15-3	OECD301F (GLP)	62.0% ThOD	**10**	Staples et al., 1999, 2001
NP	25154-52-3	OECD 301C	0% ThOD	**NR***	CITI, 1992
* Provided as supplemental information as this study does not meet the study quality criteria for relevance.					

In addition to quantifying ultimate biodegradation by measuring CO_2 production, primary biodegradation was quantified in the OECD 301B test. Dissolved organic carbon (DOC), suspended organic carbon (SOC), as well as concentrations of NP remaining at days 15 and 35 were measured. Based on DOC removal, NP/NPE degraded by 87.1 to 97.6% and conversion of the test material into cellular biomass or sorbed onto particulates was measured as SOC, and comprised 15.1% to 48.4% of the initial carbon. Concentrations of NP were measured using GC/MS on days 15 and 35 and summed for comparison to total concentrations on day 0. By day 15, based on the remaining residues, degradation of the parent material tested ranged from 91.1 to ~100%. By day 35, the amount of degradation ranged from 92.3 to ~100% of initial concentrations across all tests.

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[40] US EPA. (2000). Interim Guidance for Using Ready and Inherent Biodegradability Tests to Derive Input Data for Multimedia Models and Wastewater Treatment Plants (WWT) Models.
http://www.epa.gov/opptintr/exposure/docs/halflife.htm#eqc

The OECD 301C test listed in Table 4 above, which was reported by the Japan MITI,[41] is provided as supplemental information since the study does not meet the relevance criteria. It is important to note that discrepancies are often seen between results of OECD 301C or 302C tests (typically used by MITI) and other ready biodegradability tests (e.g., OECD 301B, 301F and analogous US EPA guidelines). Some studies have shown that inherently biodegradable compounds may not pass screening tests conducted using the OECD 301C ready biodegradability test as was the case with the CITI study which showed no biodegradation for NP. The reason for these discrepancies is thought to be due to differences in the microbial inoculum used in the studies. The majority of ready biodegradability tests conducted according to current guidelines (OECD, US EPA) use freshly collected inoculum from municipal wastewater treatment plants. In contrast, studies conducted according to OECD 301C and 302C use a mixed inoculum that has been pre-conditioned for 30 days on a medium containing glucose/peptone as the sole carbon source. Such culturing of the inoculum on easily degraded substrates is considered to result in substantial loss of microbial diversity originally present in the composite sample, and thus the environmental relevance of the preconditioned inoculum has been criticized. Recent studies by using genetic techniques (DNA molecular probes) have shown that the microbial diversity of the inoculum significantly decreases during this 30 day pre-conditioning period.[42,43] Since the results of OECD 301C tests are inconsistent with the results of other screening tests conducted using standard guidelines and conducted under GLP, as well as simulation tests with these materials and because of the unique and un-natural inoculum used in OECD 301C and 302C tests, the results of the CITI (1992) study should not be used by Washington State for categorization of the persistence of NP.

In summary, ready and inherent biodegradation studies that adhered to OECD protocols or used very similar protocols have been conducted with commercial NP. The results of these tests were used to derive the environmental half-lives in Table 3. None of the half-lives derived from studies meeting criteria for reliability, relevance and adequacy exceed Washington State's proposed half-life criteria for persistence.

Simulation Tests with Branched Test Materials

Die-Away Studies with Freshwater and Freshwater Sediments

Dutka et al. (1998) conducted shake flask tests with NP in freshwater and sediment-containing systems under either oxic or anoxic conditions. Sediments were taken from a lake near a creek from which wastewater treatment effluents flow. The sediments contain background concentrations of NP (~84 µg/g dry weight). One experiment measured the degradation of the background NP over time, while a second experiment measured NP

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[41] Chemical Inspection and Testing Institute (CITI). (1992). Biodegradation and bioaccumulation data of existing chemicals based on the CSCL Japan. Chemical Inspection and Testing Institute, Tokyo, Japan. http://www.safe.nite.go.jp/english/kizon/KIZON_start_hazkizon.html.

[42] Liu, W.T., Marsh, T.L., Cheng, H. et al. (1997). Characterization of microbial diversity by determining terminal restriction fragment length polymorphisms of genes encoding 16C rRNA. Appl. Environ. Microbiol., 63, 4516-4522.

[43] Forney, L.J., Liu, W.T., Guckert, J.B. et al. (2001). Structure of microbial communities in activated sludge: potential implications for assessing the biodegradability of chemicals. Ecotox Environ Safety, 49, 40-53.

concentrations over time in sediment spiked with NP (to a total of about 500 µg/g NP). Sediment concentrations of NP were measured over the course of 6 months. NP in unspiked sediment decreased by 89.5% in four weeks, but did not degrade further. The authors speculated that additional NP was being formed in the sediment during the test from degradation of other NPE residues present. NP in spiked sediment increased in concentration over the first three months, followed by degradation of 62.5% by the sixth month. It is likely that the apparent increasing concentrations merely represent non-homogeneity of the spiked NP in the sediments. Similar experiments were conducted under anoxic conditions. No decrease of NP was noted under anoxic conditions.[44]

Sundaram and Szeto (1981) measured the degradation of NP in freshwater-sediment mixtures. Some of the NP added to the systems became adsorbed to the sediment during the course of the study. However, by day 71, 80% had been biodegraded, while 20% remained incorporated in the sediment.[45]

Yuan et al. (2004) conducted static die-away tests on NP with river sediments.[46] A portion of the sediments was acclimated to NP. Separate studies were conducted at 30°C with unacclimated sediment, acclimated sediment, at various temperatures (20 to 50°C), and with or without shaking. For static systems at 30°C, the authors reported half-lives across four sediments of 13.6 to 99.0 days for NP. In static systems also at 30°C using both unacclimated and acclimated sediment, half-lives for NP were 20.4 and 5.1 days, respectively. The time to disappearance was 70 days for unacclimated sediment and about 27 days for acclimated sediment. Temperature was a significant factor in controlling the degradation rate of NP. For NP, half-lives of 40.8, 4.2 and 3.0 days were reported for 20°C, 40°C and 50°C. An additional important factor in the conduct of these tests was whether or not the test vessels were shaken or static. Shaking the flasks decreased the half-lives by a factor of about two to five. Absence of shaking likely helped create anoxic conditions in the sediments; NP is known to degrade slower or not at all under anoxic conditions.[47]

Die-Away Studies with Seawater and Marine Sediments

Ekelund et al. (1993) reported the results of shake flask die-away tests of synthesized ^{14}C-NP in seawater or a blend of seawater and marine sediment conducted at 11°C. With seawater only, the authors reported a 28 day lag phase followed by formation of CO_2 (55% by day 56). With seawater and sediment, no lag phase was observed and formation of CO_2 started immediately reaching 44% by day 56. The overall mass balance of this study was <50%, so it is possible that additional mineralization occurred but CO_2 was

[44] Dutka, B.J., Liu, D., Jurkovic, A., McInnis, R., Lee, H-B., Onuska, F., and Rao, S.S. (1998). Observations from a six month study on the effect of biodegradation processes in sediment on the toxicity potential of targeted chemicals. Environ. Toxicol. Water Qual., 13, 313-322.

[45] Sundaram, K.M.S., and Szeto, S. (1981). The dissipation of nonylphenol in stream and pond water under simulated field conditions. J. Environ. Sci. Health B, 16, 767-776.

[46] Yuan, S.Y., Yu, C.H., and Chang, B.V. (2004). Biodegradation of nonylphenol in river sediment. Environ. Pollut., 127, 425-430.

[47] Dutka, B.J., Liu, D., Jurkovic, A., McInnis, R., Lee, H-B., Onuska, F., and Rao, S.S. (1998). Observations from a six month study on the effect of biodegradation processes in sediment on the toxicity potential of targeted chemicals. Environ. Toxicol. Water Qual., 13, 313-322.

lost. However, it is more likely that unextractable metabolites were formed or that incorporation into cellular material occurred.[48,49]

Die-away Studies with Soil and Sludge Amended Soils

Marcomini et al. (1988, 1989) reported the results of die-away studies of NP in soil to which anaerobically digested sludge was added. NP was reduced by 80% in the first 21 days. Further reduction was negligible due to sorption of the metabolites to soil and their subsequent lack of bioavailability.[50,51]

Kubiak (2002) conducted laboratory lysimeter studies using two soil types with and without plants and monitored the leaching and degradation behavior of ^{14}C-NPE3. Dissipation half-lives ranged from <1.5 to <6 days. Lower ^{14}C-NPE and ^{14}C-NP were formed as transient products that were quickly degraded to ^{14}CO$_2$ (the actual rates were not provided in the text). Up to 20% of the applied radiolabel consisted of bound residue.[52]

Topp and Starratt (2000) examined the mineralization of ring-U-^{14}C-labeled NP in several soils as a function of temperature. ^{14}C-NP fate was assessed in microcosms incubated at either 30°C or a range of temperatures from 4 to 30°C. In all soils, an initial rapid phase of degradation occurred (30% loss of applied radioactivity in first 10 days) followed by a slower phase. The authors calculated mineralization half-lives ranging from 4.5 to 16.7 days based on the more rapid initial phase. Degradation was a function of temperature (tested at 5 to 30°C) with increasing lag times and slower overall degradation at lower temperatures (half-lives not reported). Most remaining activity was not extractable (50% of initial activity). The authors also showed that the moisture content of the soil affected the rate of degradation. Saturated soil and nearly dry soil had significantly lower capability to mineralize the NP (10 to 25% of that achieved with moist but not saturated soil). An additional test was conducted in which soils were amended with municipal sewage sludge from an aerobic treatment plant to assess the effect of the sludge on the bioavailability of NP for mineralization. The authors reported that the sludge-amended soil degraded NP rapidly, but was controlled by oxygen availability. Heavily amended soil may limit degradation of the NP due to the high oxygen demand of the sludge.[53]

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[48] Naylor, C.G., Staples, C.A., Klecka, G.M., Williams, J.B., Varineau, P.T., and Cady, C. (2005). Biodegradation of [^{14}C] ring-labeled nonylphenol ethoxylate. Archives of Environmental Contamination and Toxicology. In-press.

[49] Ekelund, R., Granmo, A., and Magnusson, K. (1993). Biodegradation of 4-nonylphenol in seawater and sediment. Environ. Pollut., 79, 59-61.

[50] Marcomini, A., Capel, P.D., and Giger, W. (1988). Residues of detergent-derived organic pollutants and polychlorinated biphenyls in sludge-amended soils. Naturwissenschaften, 75, 460-462.

[51] Marcomini, A., Capel, P.D., and Lichtensteiger, Th. (1989). Behavior of aromatic surfactants and PCBs in sludge-treated soil and landfills. J. Environ. Qual., 18, 523-28.

[52] Kubiak, R. (2002). Alkylphenols in agrar ecosystems. Second statusseminar/endocrine disrupters, pp. 93-95, UKF, BMBF, UBA, BMU.

[53] Topp, E., and Starratt, A. (2000). Rapid mineralization of the endocrine-disrupting chemical 4-nonylphenol in soil. Environ. Toxicol. Chem., 19, 313-318.

Kuchler (1996) conducted mineralization and sorption studies of NP in soil lysimeters. NP did not migrate beyond the first 10 cm depth of the soil and no NP was detected in soil or leachate after three to four weeks. Removal of all applied NPE was 99.8%.[54]

Mortensen and Kure (2003) reported the results of die-away tests of NP in soils to which sewage sludge and other organic wastes were amended. Amended soils were either planted with rape or not planted. Soil amended with anaerobic digester sludge that was planted with rape had less residual NP at 30 days than amended soil without plants (13 vs. 26%). Similar results were found with soil amended with activated sludge (8.3% in planted amended soil vs. 18% without plants). NP in soil amended with activated sludge or compost was reduced by 84% and 64%, respectively, by day 30.[55]

Dettenmaier et al. (2004) conducted similar tests examining the fate of NP in soil amended with biosolids and which were either unplanted or planted with crested wheat grass. The extent of degradation was not different between planted and unplanted microcosms. Mineralization ranged from 7.2 to 11% for NP by day 150.[56]

Jacobsen et al. (2004) conducted lysimeters studies using a sandy loam soil into which anaerobically digested sewage sludge (that contained NP) was incorporated to a depth of 15 cm. After incorporation, the NP concentration was 0.56 μg/g, dry weight. Lysimeters were placed outdoors to receive natural lighting, temperature and precipitation. Lysimeters were also irrigated with a constant volume of water. Samples of leachate and soil from three layers were collected periodically during the 110 day experiment and analyzed for NP. The authors reported rapid die-away of NP in the first 10 days (55% reduction). A slower but consistent reduction occurred throughout the remainder of the experiment (to 110 days). The half-life of the degradation of NP over days 10 to 110 was calculated to be 37 days.[57]

Dubroca et al. (2005) measured the degradation of a mixture of linear and branched ^{14}-C-U-NP in an agricultural soil. By day 8, about 40% of initial radiolabeled test material was bound to the soil. By day 64, about 30% of initial radiolabel was collected as carbon dioxide. The remaining approximately 30% of initial ^{14}C-NP was present as extractable material. HPLC analyses showed that NP was present at negligible concentrations by day 32. From these data, the authors calculated biodegradation half-lives of 4 days for the mix of linear and branched.[58]

[54] Kuchler, T. (1996). Behavior of surfactants and their influence on the mobility of organic micropollutants in sandy soils with weak sorption properties. Ph.D. Thesis, University of Potsdam, Germany.

[55] Mortensen, G.K., and Kure, L.K. (2003). Degradation of nonylphenol in spiked soils and in soils treated with organic waste product. Environ. Toxicol. Chem., 22, 718-721.

[56] Dettenmaier, E.M., Chard, J.K., and Doucette, W.J. (2004). Fate of alkylphenol-polyethoxylates in soil/biosolids systems planted with crested wheatgrass. Society of Environmental Toxicology and Chemistry, 25th Annual Meeting, Portland, OR.

[57] Jacobsen, A.M., Mortensen, G.K., and Hansen, H.C.B. (2004). Organic compounds in the environment. Degradation and mobility of linear alkylbenzene sulfonate and nonylphenol in sludge-amended soil. J. Environ. Qual., 33, 232-240.

[58] Dubroca, J., Brault, A., Kollmann, A., Touton, I., Jolivalt, C., Kerhoas, L., and Mougin, C. (2005). Biotransformation of nonylphenol surfactants in soils amended with contaminated sewage sludges. Environmental Chemistry: Green Chemistry and Pollutants in Ecosystems, pp. 305-315.

<u>Field Studies</u>

Heinis et al. (1999) reported the results of a field study in which NP was added every two days over a 20 day period to an artificial enclosure of a littoral zone in a small lake. Samples of water, sediment and plant tissue were periodically measured for NP content over the course of 440 days. Dissipation half-lives (includes degradation and sorption) averaged 0.74 day in surface water, 66 days in sediments and 10 days in plants. The authors did not distinguish between amounts of water-borne NP that was biodegraded and that adsorbed to sediments, macrophytes or soil. However, the authors did calculate an approximate mass balance of all applied NP for two of the enclosures. Initially all NP was in the water column. By day two after application, approximately 10% was sorbed to enclosure walls (8.3 to 8.7%), macrophytes (1.2 to 1.4%) and sediment (0.7%). The rate of dissipation from the water column increased during the 20 day dosing period so proportionally the amounts sorbed to the walls, plants and sediment increased. However, the absolute amounts continued to decrease. Following the dosing period of 20 days, the amount of applied NP that was sorbed to the sediment never exceeded about 1%. It was assumed that the sorbed NP to the enclosure walls desorbed back into the water over time. NP sorbed to the plants either degraded or desorbed to the water. The fact that only relatively minor amounts of applied NP were present in the sediment at any time suggests that biodegradation was the dominant removal process in the enclosures. Thus the dissipation half-life of NP in the water of 0.74 days is effectively a biodegradation half-life.[59]

Jonkers et al. (2003) reported on biodegradation of NPE and their degradation intermediates in field studies of estuarine sediment. Samples were collected in two estuaries, the first was stratified with a short retention time (1 to 3 days) and the second with a retention time of two to three months. Sources of NPE were wastewater treatment plants upstream of the estuaries. Despite the relatively short retention time in the first estuary, NPE steadily decreased forming mainly NPEC and negligible NP. Due to stratification of salinity and the relatively short retention time of water flowing in the estuary, the authors could not conclude that biodegradation occurred. However, in the second estuary, NPE steadily decreased with distance from the source with NPEC>3, NPE, and NP decreasing faster than the formation and subsequent disappearance of NPEC1,2. No CAPEC were detected. The authors conclude that these data show evidence of biodegradation in the field and support laboratory results as well.[60]

Kuchler et al. (1996) examined the fate of NPE in soils in field trials. During a one year period, 10 soil plots were treated with two different sewage sludges that contained residues of NPE and NP. After application, the sewage sludge was incorporated into the top 5 cm of the soil. Soil samples were collected from various depths (0-10 cm, 10-20 cm and 20-30 cm) and analyzed for the presence of NPE and NP. NPE concentrations

[59] Heinis, L.J., Knuth, M.L., Liber, K., Sheedy, B.R., Tunell, R.L., and Ankley, G.T. (1999). Persistence and distribution of 4-nonylphenol following repeated application to littoral enclosures. <u>Environ. Toxicol. Chem.</u>, <u>18</u>, 363-75.

[60] Jonkers, N., Knepper, T.P., and DeVoogt, P. (2001). Aerobic biodegradation studies of nonylphenol ethoxylates in river water using liquid chromatography-electrospray tandem mass spectrometry. <u>Environ. Sci. Technol.</u>, <u>35</u>, 335-340.

rapidly decreased, with no compound being detected after 20 days. No leaching of NPE or NP was seen from the top (0-10 cm layer) indicating that removal was by biodegradation. Although NP initially increased in concentration during the first 10 days, which indicates that it was possibly formed from the degradation of NPE, but no NP was detected after 20 days, indicating that it was degraded.[61]

Gross et al. (2004) traced the fate of APE1-3, AP, and combined APEC1-3 and CAPEC1-3 in a river and wetlands. The substances originated from four wastewater treatment plants located on the river or on creeks that discharged into the wetlands. The river flowed into the wetlands and the outflow of the wetlands returned to the river. Water samples were taken immediately after the treatment plant outfall and 10 km downstream. The authors calculated that median concentrations of AP, APE1-3, and APEC1-3 combined with carboxylated APEC1-3 decreased by 92%, 95%, and 85%, respectively. The median concentration of all analyses combined decreased by 86%. Between wetland inflow and outflow, median concentrations of AP declined by 75%, however, combined APEC1-3+CAPEC1-3 decreased only by 8%. APE were not detected in either inflow or outflow. The median concentration of all analytes combined decreased by 11%. In the samples taken immediately downstream of the discharge, APEC1-3+CAPEC1-3 comprised 83% of all analytes, while 10 km downstream they comprised 99% of all analytes. Median concentrations of CAPEC1-3 were approximately two to seven times that of the APEC1-3. At the outflow to the wetlands, nearly all of the substances are as APEC1-3+CAPEC1-3, which are acidic substances. The authors suggested that both the river and the wetland were highly effective at removing AP and APE and that the river effectively removed APEC+CAPEC, but that the wetland treatment was poor at attenuating APEC and CAPEC.[62]

Huntsman et al. (2005) examined the fate of NPE9 (commercial detergent product) discharged into an on-site wastewater disposal (septic) system, which also evaluated the biodegradation intermediate NP. NPE9 based detergents were metered daily into the plumbing at a single-family household. The ethoxylate-containing wastewater was discharged into the highly anoxic environment of a 4500 L septic tank prior to distribution into the oxic subsurface via 100 meters of leach line. Soil pore water and ground water samples were collected and analyzed for NPE, NPEC, and NP. NP, which is not presumed to be present at greater than trace amounts in the parent NPE, was detectable in the tank effluent. From there to the lower lysimeters that gather pore water, NP was degraded by 99.96%. The NPE9 and the degradation intermediates that were measured were reduced by 99.99%. The results indicate that degradation of the surfactant occurs within the anoxic portion of the disposal system followed by rapid biodegradation in the oxic unsaturated zone. These results show that NPE rapidly and completely degrades in on-site wastewater disposal (septic) systems.[63]

[61] Kuchler, T. (1996). Behavior of surfactants and their influence on the mobility of organic micropollutants in sandy soils with weak sorption properties. Ph.D. Thesis, University of Potsdam, Germany.

[62] Gross, B., Montgomery-Brown, J., Naumann, A., and Reinhard, M. (2004). Occurrence and fate of pharmaceuticals and alkylphenol ethoxylate metabolites in an effluent-dominated river and wetland. Environ. Toxicol. Chem., 23, 2074-83.

[63] Huntsman, B.E., Staples, C.A., Naylor, C.G., and Williams, J.B. (2005). Treatability of nonylphenol ethoxylate surfactants in on-site wastewater disposal systems. Water. Environ. Res. In-press.

Persistence - Summary and Conclusions

Biodegradation - the dominant removal process of NP and its ethoxylate from water, sediment, and soil - has been extensively studied and numerous studies have been conducted that focus on both the primary and ultimate degradation these compounds. Three main types of biodegradation studies have been performed. The first are stringent screening tests that measured the ready biodegradability of a test substance. The second are simulation tests in which biodegradation is measured in laboratory tests using procedures and bacterial inocula that simulate potential degradation in specific environmental compartments or within wastewater treatment plants. Simulation tests for the biodegradation of NP are available for all relevant environmental compartments including freshwater, freshwater sediment, seawater, marine sediment and soil. The third line of evidence that biodegradation is the key removal process for NP and their biodegradation intermediates is based on results of field studies.

The ready biodegradability of NP has been measured using standard OECD 28-day protocols conducted under GLP. Using EPA guidance on extrapolating environmental half-lives based on the results of ready biodegradation tests, half-lives in water were determined. The results are presented in Table 4 and show that none of the test substances meet the proposed Washington State criteria for persistence.

Half-lives for biodegradation within specific environmental compartments were also taken from the simulation studies that were reviewed above. Half-lives were either calculated by the authors or were calculated here if there were sufficient data to do so. The compiled biodegradation half-lives from studies that used the commercially relevant branched materials are shown in Table 5 below.

**Table 5. Summary of Biodegradation Half-lives from Ready Tests,
Laboratory Simulation Data, and Field Studies (All Media) for Studies
that Used Commercially Relevant (Branched) NP**

Compound	Basis of decision	Media	Half-life (days)	Reference
NP	Ready test	Water Soil Sediment	5 to 10 5 to 10 20 to 40	Staples et al., (1999, 2001)
NP	River die-away	Water	40.8	
NP	River die-away	Water	2.5 to 35	
NP	Seawater die-away	Water	26.3	
NP	Sediment die-away	Sediment	26.3	
NP	Seawater die-away	Sediment	5.9	
NP	Soil die-away	Soil	10.4	
NP	Sludge-amended soil	Soil	7.3 to 9.5	
NP	Loamy sand die-away	Soil	37	Jacobsen et al., (2004)
NP	Soil die-away	Soil	4.5 to 16.7	Topp and Starratt (2000)

Half-lives for NP in river water ranged from 2.5 to 40.8 days. In seawater and marine sediments, die-away half-lives were 5.9 and 26.3 days, respectively. In soil, biodegradation half-lives for NP ranged from 4.5 to 37 days. <u>None of the half-lives</u>

measured on commercially relevant branched NP exceed the proposed Washington State persistence criteria.

In conclusion, the fate and persistence of NP in the environment is well understood and has been studied in all compartments where it may be expected to be found. The database examining the fate and persistence of NP includes stringent screening tests, laboratory simulation tests and field studies, which collectively and conclusively show that this compound should not be categorized as persistent.

IV. BIOACCUMULATION

Department of Ecology Review of Bioaccumulation

Ecology's Review of bioaccumulation potential for NP consists of the following short paragraph.

> *Published K_{ow} values for nonylphenol (K_{ow} values range from 4.2 - 6) indicate there is a potential for bioaccumulation in aquatic organisms and humans. The European Union (ECB, 2002) reviewed information on bioaccumulation of NP and concluded "...[i]t is clear from the available data that nonylphenol bioconcentrates to a significant extent in aquatic species..." and used a BCF of 1,280 to characterize the bioaccumulation potential of this substance. Other recent evaluations have used a range of BCF values to characterize the bioaccumulation potential of NP compounds. For example, EPA (1998b) used a BCF of 1,288 for 4-NP, 550 for NP and 7,079 for 4-NP (branched)); the OSPAR Commission (2002) reported BCF values ranging from 280-4,120 for 4-NP and used a BCF of 1,280 (from ECB (2002)) for NP and 4-NP(branched); EPA (2003) reported lipid normalized BCF values ranging from 39-209 (freshwater fish) and 78.75 to 2,168 (salt water organisms). Environment Canada (2000) reported the same range of BCF values and concluded that "...the available literature suggests that the ability of NP and NPEs to bioaccumulate in aquatic biota in the environment is low to moderate...". The PBT Profiler (EPA, 2004c) calculates the following BCF value using the BCFWin computer program: 540 (4-NP); 540 (NP) and 7,200 (4-NP (branched).*

As discussed previously, bioconcentration factor (BCF) and bioaccumulation factor (BAF) data from studies that meet criteria for reliability, relevance and adequacy should be given more weight than calculated or modeled values in assessments to characterize the bioaccumulation potential of a compound. The US EPA Waste Minimization Prioritization Tool (WMPT)[64] was used to derive the BCF values 1,288 and 7,079 listed in Ecology's bioaccumulation review paragraph and the EPA PBT profiler model[65] was used to calculate BCF values various NP isomers that ranged from 550 to 7,200. The

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[64] US EPA. (1998, September). Waste Minimization Prioritization Tool Spreadsheet Document for the RCRA Waste Minimization PBT Chemical List Docket (# F-98-MMLP-FFFFF).
[65] US EPA. (2004b). PBT Profiler. Prepared by Environmental Science Center for the Office of Pollution Prevention and Toxic Substances, US EPA. Version 1.203.

BCF value of 1,280 cited in the Department of Ecology review paragraph was calculated for use in the EU risk assessment on NP using a log Kow of 4.48. In light of the availability of high quality bioaccumulation studies, Ecology should not rely on the BCF values calculated or derived using models. In fact the range of results predicted using EPA's PBT Profiler provide a good example of the potential uncertainty in BCF values generated by a model for various NP isomers and highlight the point that measured values on commercial material provide a more accurate understanding of bioaccumulation potential. In addition, the same EU Risk Assessment document that calculated the BCF value of 1,280 stated that measured BCFs (fresh weight basis) of up to 1300 "may overestimate the BCF; more reliable values with a mean of 741 have been measured, which are a similar order of magnitude."[66]

The BCF values of 2,168 and 4120, which are listed in the Ecology's review paragraph on the bioaccumulation of NP were originally taken from Ekelund *et al*, 1990.[67] This study should be ranked as "use with caution" since the authors of the original study noted that the BCF values are based on total ^{14}C measurements thus the presence of radiolabeled metabolites in the organisms may have led to an overestimate of the bioconcentration potential. In fact, the finding of radiolabeled metabolites of NP along with a finding that NP depuration in fish was complete in five days in the Ekelund et al., 1990 study support the conclusion that NP is not bioaccumulative. Ecology should not favor these estimated BCF values from this "use with caution" study when other BCF values from more reliable studies are available. Many of these lower BCF values are also cited along with the Ekelund data in EPA's Proposed WQC for NP, which concluded only that "nonylphenol bioaccumulates in aquatic organisms to low levels."[68]

Bioaccumulation - Other Readily Available Data That Ecology Should Consider

When data are available for different classes of species (e.g., algae, water fleas and fish), the BAF or BCF data for the fish species should be used to represent the bioaccumulation behavior of the chemical substances for the purpose of bioaccumulation categorization.[69] The rationale for the preference of fish data over those for lower trophic levels is that the potential for chemical bioaccumulation in the food chain is best measured by the BAF or BCF in higher trophic level organisms. Consequently, in view of the extensive information available, the following discussion will focus on the BAF and BCF potentials in fish.

As summarized in Tables 6 and 7 numerous studies with fish are available to inform the decision on the BAF and BCF potential for the NP. The subject has also been reviewed

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[66] European Union. (2001). Risk Assessment Report: 4-Nonylphenol (branched) and Nonylphenol: Final Report. http://ecb.jrc.it/DOCUMENTS/Existing-Chemicals/RISK_ASSESSMENT/REPORT/.

[67] Ekelund, R., Bergman, A., Granmo, A., and Berggren, M. (1990) Bioaccumulation of 4-nonylphenol in marine animals - a re-evaluation. Environmental Pollution, 64, 107-112.

[68] US EPA. (2003). Ambient Aquatic Life Water Quality Criteria for Nonylphenol - Draft. EPA 822-R-03-029. http://www.epa.gov/waterscience/criteria/nonylphenol/draft-nonylphenol.pdf

[69] Environment Canada. (2003). Environment Canada Guidance Manual for categorization of Organic and Inorganic Substances on Canada's Domestic Substances List.

in several publications.[70,71,72] Note that some studies have specified the CAS number of the test chemical, while other have only indicated whether the test material was linear or branched NP. While several studies are judged "valid" or "reliable" for assessing BAF and BCF potential, the other studies, which provide results from shorter exposure periods or field investigations, are useful in contributing to the overall weight of evidence.

<u>Laboratory studies</u>

The bioconcentration of NP (technical grade, branched) in juvenile Atlantic salmon (*Salmo salar*) was studied by McLeese et al. (1981) with static exposure studies conducted over a period of 4 days. Given the short exposure period and the static exposure conditions, the study should be judged use with caution. The uptake rate constant was measured to be 45 mL-g^{-1}-day^{-1} and the excretion rate constant was 0.16 day^{-1}, giving a steady-state bioconcentration factor of around 280 mL/g wet weight. The excretion half-life was estimated to be about four days.[73]

Ekelund et al. (1990) studied the bioaccumulation of ^{14}C-labeled NP in three-spined stickleback (*Gasterosteus aculeatus* L.). The ^{14}C-labelled p-NP was synthesized from uniformly labeled phenol and unlabelled nonene. Analysis of the synthetic material indicated the isomer distribution was consistent with commercial NP. The animals were exposed to 4.8-4.9 micrograms/L ^{14}C-NP in a flow-through system. Exposure was for 16 days followed by an elimination period of 32 days. The authors found that steady state bioaccumulation had been reached by the end of the exposure period, and that NP was then rapidly eliminated from the fish. Bioconcentration factors for the fish were in the range of 1,200 to 1,300 mg/L on a fresh weight basis. The authors noted that the BCF values are based on total ^{14}C measurements in fish tissue, thus, the presence of metabolites in the organisms may have led to an overestimate of the bioconcentration potential.[74] The fact that steady state conditions were achieved, but that BCF values were based on total fish radioactivity, supports classifying this study as use with caution.

The bioconcentration of NP (84852-15-3, commercial product) in the fathead minnow (*Pimephales promelas*) was investigated by Ward and Boeri (1991).[75] The study was conducted according to US EPA test guidelines in accordance with Good Laboratory Practices, and is considered valid for the purposes of categorization. Fathead minnows (0.5-1 g) were exposed to separate nominal concentrations of 5 micrograms/L and 25 micrograms/L NP in an intermittent flow-through system for 20 days. The exposure

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[70] Staples, C.A., Weeks, J., Hall, J.F., and Naylor, C.G. (1998). Evaluation of aquatic toxicity and bioaccumulation of C8- and C9-alkylphenol ethoxylates. <u>Environ. Toxicol. Chem.</u>, <u>17(12)</u>, 2470-2480.

[71] Servos, M.R. (1999). Review of the aquatic toxicity, estrogenic responses and bioaccumulation of alkylphenols and alkylphenol polyethoxylates. <u>Water Qual. Res. J. Canada</u>, <u>34</u>, 123-177.

[72] European Union. (2001). Risk Assessment Report: 4-Nonylphenol (branched) and Nonylphenol: Final Report. <u>http://ecb.jrc.it/DOCUMENTS/Existing-Chemicals/RISK_ASSESSMENT/REPORT/</u>.

[73] McLeese, D.W., Zitko, V., Sergeant, D.B., Burridge, L., and Metcalfe, C.D. (1981). Lethality and accumulation of alkylphenols in aquatic fauna. <u>Chemosphere</u>, <u>10</u>, 723-730.

[74] Ekelund, R., Bergman, A., Granmo, A., and Berggren, M. (1990). Bioaccumulation of 4-nonylphenol in marine animals - a re-evaluation. <u>Environ. Pollut.</u>, <u>64</u>, 107-120.

[75] Ward, T.J., and Boeri, R.L. (1991). Bioconcentration test with nonylphenol and the fathead minnow Pimephales promelas. Chemical Manufacturers Association, Washington, DC.

period was then followed by a 7-day depuration period. The system was analyzed for NP concentrations, dissolved oxygen content, temperature, and pH. Measured levels of NP were 4.9 micrograms/L and 22.7 micrograms/L in the two test systems, respectively. The concentration of NP in fish tissues increased from background concentrations to steady state concentrations during the first 3-10 days of exposure, a finding that was consistent with previous bioconcentration studies with NP. Uptake and depuration of NP appeared to be independent of the concentration of the test substance in water. Exposure of fathead minnows to 4.9 micrograms/L NP in water for 20 days resulted in a BCF of 271 L/kg fresh weight with an uptake rate constant of 133 mL-g^{-1}-day^{-1} and a depuration rate constant of 0.49 day^{-1}. Exposure to 22.7 micrograms/L NP for 20 days resulted in a BCF of 344 L/kg fresh weight with an uptake rate constant of 193 mL-g^{-1}-day^{-1} and a depuration rate constant of 0.56 day^{-1}. In summary, flow-through exposures of NP with fathead minnows conducted in accordance with US EPA guidelines produced steady-state BCF values of approximately 300 mL/g, with uptake rate constants of ~150 mL-g^{-1}-d^{-1} and depuration rate constants of 0.5 day^{-1}; the depuration rate constants are consistent with an elimination half-life for NP from fathead minnows of approximately 1.5 days.

CITI (1992) reported bioconcentration factors for carp (*Cyprinus carpio*) exposed to measured concentrations of linear NP (25154-52-3) in separate exposures at 100 micrograms/L and 10 micrograms/L in a flow through system.[76] According to the EU risk assessment, the test is considered valid for the determination of the bioconcentration factor (EU, 2003). The carp were exposed to linear NP for eight weeks. At the 100 micrograms/L exposure concentration, bioconcentration factors of 250-330 were measured over the eight week period. At the 10 micrograms/L exposure level, bioconcentration factors ranged from 90 to 220. These results are similar to those observed in an US EPA-guideline study (Ward and Boeri, 1991) with fathead minnows (a member of the carp family) and the results collectively indicate that NP has a low potential for bioaccumulation in fish.[77]

Brooke (1993) determined bioconcentration factors for fathead minnow (*Pimephales promelas*) and bluegills (*Lepomis macrochirus*) over 28 days with exposure to 5 separate concentrations of NP (84852-15-3).[78] Results of the study are considered valid for the purposes of categorization. For fathead minnows exposed to concentrations of 9.3, 19.2, 38.1, 77.5 and 193 micrograms/L, the mean BCF ($\pm$ standard deviation) value was 586 $\pm$ 273 mL/g wet weight after 14 days exposure and 741 $\pm$ 206 mL/g wet weight after 28 days exposure. The BCF with fathead minnows was found to be independent of NP concentration at 28 days but not at 14 days; reduced growth of the fish was seen at the two highest concentrations tested. For bluegill exposed to NP concentrations of 5.6, 12.4, 27.6, 59 and 126 micrograms/L, the mean BCF ($\pm$ standard deviation) value was 262$\pm$70 mL/g wet weight after 14 days and 220 mL/g wet weight after 28 days. The BCF for this

[76] Chemical Inspection and Testing Institute (CITI). (1992). Biodegradation and bioaccumulation data of existing chemicals based on the CSCL Japan. Chemical Inspection and Testing Institute, Tokyo, Japan. http://www.safe.nite.go.jp/english/kizon/KIZON_start_hazkizon.html.

[77] Ward, T.J., and Boeri, R.L. (1991). Bioconcentration test with nonylphenol and the fathead minnow Pimephales promelas. Chemical Manufacturers Association, Washington, DC.

[78] Brooke, L.T. (1993). Acute and chronic toxicity of nonyphenol to ten species of aquatic organisms. EPA report 68 C-0034. US EPA, Duluth, MN.

species was found to be independent of exposure concentration at the three lowest concentrations, but the BCF at the two higher concentrations was found to be lower, particularly at 14 days, than that obtained at the lower concentrations.

Lewis and Lech (1996) studied the uptake, disposition and persistence of ^{14}C-NP from water in the rainbow trout (*Oncorhynchus mykiss*).[79] The bioconcentration of ^{14}C-NP in the trout was determined by exposing juvenile fish weighing 40-60 g under static conditions to 18 micrograms/L ^{14}C-nonylphenol (uniformly ring labeled) for 5-24 hours. The calculated bioconcentration factors were 24 mL/g for the whole fish after 5 hours exposure and 98 for the viscera after 24 hours exposure. Given the limited exposure period, the study should be used with caution. To investigate the disposition of ^{14}C-NP in the trout, juvenile fish weighing 40-60 g were exposed under static conditions to 36 micrograms/L ^{14}C-NP for 14 hours. The test chemical was detected in the following tissues in descending order of concentration: bile, liver, kidney, fat, gill, heart, and muscle. The half-life of ^{14}C-nonylphenol in specific tissues was determined by exposing juvenile fish weighing 40 to 60g under static conditions to 18 micrograms/L ^{14}C-NP for 8 hours. The half-life was calculated as 19.8 hours in fat, 18.6 hours in muscle, and 5.9 hours in liver; these dissipation half-life values for NP in fish are consistent with previous research. To investigate the metabolism of ^{14}C-NP in the fish, bile from exposed fish was analyzed and shown to contain three glucuronide metabolites, indicating phase II metabolism of NP in rainbow trout.

Giesy et al. (2000) determined bioconcentration factors for adult fathead minnow (*Pimephales promelas*) following 42 days exposure to five separate concentrations of NP (commercial product, branched).[80] NP concentrations in water were measured at the beginning, middle, and end of the experiments. For fathead minnows exposed to concentrations of 0.05, 0.16, 0.4, 1.6 and 3.4 micrograms/L, the BCF (on a wet weight basis) values ranged from 203 to 268 after 42 days, indicating that BCF was independent of the exposure concentration. The test material was branched NP obtained from one of the manufacturers of commercial NP (Schenectady International). As indicated in Table 6, the experimental results of Giesy et al. (2000) have been assigned to CAS number 84852-15-3.

The bioconcentration of branched NP in the killifish (*Oryzias latipes*; medaka) was investigated by Tsuda et al. (2001).[81] The fish (0.16-0.24 g wt) were exposed to measured NP concentrations of 3.6 ± 0.9 micrograms/L NP in a flow-through system for 7 days. The exposure period was then followed by a 1-day depuration period. BCF values in whole fish (wet weight) reached a plateau after 48 hours of exposure, suggesting that steady state conditions were established; hence the study has been judged use with caution. Exposure of the minnows to 3.6 micrograms/L NP in water for 7 days

[79] Lewis, S.K., and Lech, J.J. (1996). Uptake, disposition, and persistence of nonylphenol in rainbow trout. Xenobiotica, 26, 813-819.
[80] Giesy, J.P., Pierens, S.L., Snyder, E.M., Miles-Richardson, S., Kramer, V.J., Snyder, S.A., Nichols, K.M., and Villeneuve, D.A. (2000). Effects of 4-nonylphenol on fecundity and biomarkers of estrogenicity in fathead minnows (Pimephales promelas). Environ. Toxicol. Chem., 19(5), 1368-1377.
[81] Tsuda, T., Takino, A., Muraki, K., Harada, H., and Kojima, M. (2001). Evaluation of 4-nonylphenols and 4-tert-octylphenol contamination of fish in rivers by laboratory accumulation and excretion experiments. Wat. Res., 35, 1786-1792.

resulted in a median BCF of 167 $\pm$ 23 mL/g-wet weight. Following transfer to clean water the excretion rate constant and biological half-life were determined to be 0.07 h^{-1} and 9.9 h, respectively.

Smith and Hill (2004) have recently examined the uptake and metabolism of technical NP in the roach (*Rutilus rutilus*).[82] Branched ^{14}C-NP was synthesized using procedures consistent with the manufacture of the commercial product. Sexually mature roach were exposed to 4.9 $\pm$ 1.1 micrograms NP/L in a flow through system. A 4-day exposure period was chosen for the study since prior work had shown that NP concentrations in the tissues of trout reached steady state after 4 days, findings which are consistent with previously cited work. Bioconcentration factors for NP in various fish tissues were determined; however, a BCF value for whole fish was not calculated. The concentration of NP residues was the highest in bile and liver, with apparent BCF tissue values of 34,121 and 605, respectively. In the other tissues, apparent BCF tissue values ranged between 13 and 250. A single major metabolite of NP was present in liver and bile, which was identified as the glucuronide conjugate of 4-(hydroxyl-nonyl)-phenol.

Table 6 below provides a summary of Experimental Determination of Bioconcentration/Bioaccumulation Potential for NP in Fish - Lab Studies. The only measured BCF value in fish that slightly exceeds the proposed Washington State BCF criteria of 1000 were reported in the Ekelund et al (1990) study. As noted previously, the study is listed as "use with caution" and the authors noted that the BCF values are based on total ^{14}C measurements in fish tissue, thus, the presence of metabolites in the organisms may have led to an overestimate of the bioconcentration potential.

Table 6. Summary of Experimental Determination of Bioconcentration/Bioaccumulation Potential for NP in Fish - Lab Studies

Test Chemical	CAS RN	Species	Experimental Design	BCF (mL/g wet wt)	Rate Constants and Half-lives	Comment	Reference
NP Technical, branched	Not specified	Atlantic salmon *Salmo salar*	4-d test Static 170-310 ug/L	75-235 280 (k_1/k_2)	45 mL-g^{-1}-d^{-1} (uptake) 0.16 d^{-1} (depuration) 4 d (depuration t$_{1/2}$)	Use with caution BCFs were measured (non-equilibrium) and calculated (from k_1/k_2; equilibrium)	McLeese et al., 1981
^{14}C-NP Analysis showed consistent with commercial NP	Not specified	Threespine stickleback *Gasterosteus aculeatus*	16-d exposure 32-d elimination Flow-through 4.8-4.9 ug/L	1200-1300 1333 (k_1/k_2)	Half-life = 16 hr Time to 90% steady-state = 2.2 days	Use with caution depuration complete in 5 days BCF based on ^{14}C-NP (>80% in fish, analyzed via TLC)	Ekelund et al., 1990

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[82] Smith, M.D., and Hill, E.M. (2004). Uptake and metabolism of technical nonylphenol and its brominated analogues in the roach (Rutilus rutilus). Aquatic Toxicol., 69, 359-369.

NP Branched	84852-15-3	Fathead minnow *Pimephales promelas* Juvenile	US EPA Guideline, GLP 20-d exposure 7-d elimination Flow-through [2] exposure concs (4.9 and 22.7 ug/L)	271 344	133 mL-g^{-1}-d^{-1} (uptake) 0.49 d^{-1} (depuration) 1.4 d (depuration t$_{1/2}$) 193 mL-g^{-1}-d^{-1} (uptake) 0.56 d^{-1} (depuration) 1.2 d (depuration t$_{1/2}$)	Valid (GLP) Steady state reached during first 3-10 days Depuration was rapid	Ward and Boeri, 1991a
NP Linear	25154-52-3	Carp *Cyprinus carpio*	MITI Guideline 56-d test [2] exposure concs (10 and 100 ug/L)	90-220 250-330		Valid	CITI, 1992
NP Branched	84852-15-3	Bluegill sunfish *Lepomis macrochirus*	28-d test [5] exposure concs (5.6, 12.4, 27.6, 59, 126 ug/L)	220		Valid rapid depuration	Brooke, 1993
NP Branched	84852-15-3	Fathead minnow *Pimephales promelas*	28-d test [5] exposure concs (9.3, 19.2, 38.1, 77.5, 193 ug/L)	741		Valid rapid depuration	Brooke, 1993
^{14}C-NP	Not specified	Rainbow trout *Oncorhyncus mykiss* Juvenile	5-24 h exposures Static 18 ug/L NP	24 (carcass) 98 (vicera)		Use with caution BCF based on total ^{14}C Three glucuronide metabolites detected	Lewis and Lech, 1996
NP Commercial product, branched	84852-15-3 (See text)	Fathead minnow *Pimephales promelas* Adult	42-d exposure Flow-through [5] exposure concs (0.05, 0.16, 0.4, 1.6, 3.4 ug/L	203-268		Valid	Giesy et al., 2000
NP Branched	Not specified	Killifish; medaka *Oryzias latipes*	7-d exposure 1-d elimination Flow-through 3.6 ug/L	167	1.68 d^{-1} (depuration) 9.9 h (depuration t$_{1/2}$)	Use with caution steady state reached after 48 h	Tsuda et al., 2001
^{14}C-NP Branched, consistent with commercial product	Not specified	Roach *Rutilus rutilus* Adult	4-d exposure Flow-through 4.9 ug/L	13-250 (various soft tissues)		Use with caution glucuronide metabolite identified	Smith and Hill, 2004

Field studies

In addition to laboratory studies, the bioaccumulation of NP has also been examined in several field experiments. While laboratory tests involving water exposures conducted under controlled conditions are considered as providing key information on bioconcentration, field studies are considered as providing information on bioaccumulation potential since exposures may arise from both food and water. Thus,

field studies contribute to the weight of evidence for evaluating the bioaccumulation potential for classification and risk assessment purposes.

Ahel et al. (1993) studied the bioaccumulation potential of NP in freshwater organisms in the Glatt River and one of its tributaries in Switzerland.[83] The average concentration of NP in the river was 3.9 micrograms/L. NP concentrations in the tissues of fish collected from the river were as follows: *Squalius cephalus*, muscle 0.18 mg/kg dry weight, gut 0.46-1.2 mg/kg dry weight, liver 1.0-1.4 mg/kg dry weight, gills 0.98-1.4 mg/kg dry weight; *Barbus barbus* L., muscle 0.38 mg/kg dry weight, gut 0.05 mg/kg dry weight, liver 0.98 mg/kg dry weight, gills <0.03 mg/kg dry weight, heart 0.30 mg/kg dry weight, roe 0.09 mg/kg dry weight; *Oncorhynchus mykiss*, muscle 0.15 mg/kg dry weight, gut 1.6 mg/kg dry weight. Based upon the average concentration of NP in water, the BAF for fish was calculated in the range of 13 to 408 L/kg dry weight on an individual organ basis. Since NP concentrations in the fish were lower than those detected in algae, the authors concluded that negligible bioaccumulation of NP was occurring through the food chain.

To convert the field BAF values reported by Ahel et al. (1993) to units comparable with the other BCF data, the reported dry weight concentrations were recalculated on a fresh (or wet) weight basis by assuming that fish muscle was 85% water and 15% dry matter.[84] As reported in Table 7 the resulting non-lipid based fresh weight BAF values for NP for the three species ranged from 6 to 15 L/kg.

Liber et al. (1999) exposed bluegill sunfish to NP in a series of 18 aquatic mesocosms (littoral enclosures) in northeastern Minnesota.[85] The test chemical, high purity NP (96.4%, branched isomers, Schenectady International), was applied to the enclosures every two days over a 20-day period at dose levels of 3, 30, 100 and 300 micrograms/L. NP concentrations were measured in the water column and in fish, plants, and sediments. Based on the results, the calculated bioaccumulation factor for NP in bluegill sunfish was 87 ± 124 L/kg wet weight.

Tsuda et al. (2000) examined the bioaccumulation potential of NP in ayu fish (*Plecoglossus altivelis*) from three rivers flowing into Lake Biwa (Japan).[86] NP was detected in all samples of river water, with median concentrations of 0.14 to 3.08 micrograms/L. NP concentrations in whole fish collected from the rivers ranged from 10 to 110 mg/kg wet weight. Based on NP concentrations in the river water and fish, a BAF for NP in the ayu fish of 21 ± 15 L/kg was calculated.

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[83] Ahel, M., McEvoy, J., and Giger, W. (1993). Bioaccumulation of the lipophilic metabolites of nonionic surfactants in freshwater organisms. Environ. Pollut., 79, 243-248.

[84] Staples, C.A., Weeks, J., Hall, J.F., and Naylor, C.G. (1998). Evaluation of aquatic toxicity and bioaccumulation of C8- and C9-alkylphenol ethoxylates. Environ. Toxicol. Chem., 17(12), 2470-2480.

[85] Liber, K., Gangl, J.A., Corry, T.D., Heinis, L.J., and Stay, F.S. (1999). Lethality and bioaccumulation of 4-nonylphenol in bluegill sunfish in littoral enclosures. Environ. Toxicol. Chem., 18(3), 394-400.

[86] Tsuda, T., Takino, A., Kojima, M., Harada, H., Muraki, K., and Tsuji, M. (2000). 4-Nonylphenols and 4-*tert*-octylphenol in water and fish from rivers flowing into Lake Biwa. Chemosphere, 41(5), 757-762.

Hu et al. (2005) have recently studied the trophodynamic behavior of NP and NP ethoxylates in a marine aquatic food web from Bohai Bay, North China.[87] NP and NPEO concentrations were measured in 14 marine species. Co-analysis of DDT and DDT metabolites in of all the samples allowed direct comparison of the bioaccumulation behavior of DDT with that of NP and NPEO. The lipid equivalent concentrations of several DDT metabolites (DDE and DDMU) increased with increasing trophic level as determined by stable isotope ratios. Trophic magnification factors for DDE and DDMU were 3.26 and 3.7, respectively. In contrast, lipid equivalent concentrations of NP and all of the NPEO did not exhibit a statistically significant correlation with trophic levels in the food web. Trophic magnification factors for NP and NPEO ranged from 0.45 to 1.22. These results show that trophic magnification of NP and NPEO is not expected.

Table 7. Summary of Experimental Determination of Bioconcentration/Bioaccumulation Potential for NP in Fish - Field Studies

Chemical	Species	Experimental Design	Observations	Field BAF L/kg –wet wt	Reference
NP	Rainbow trout *Oncorhyncus mykis* Barbel *Barbus barbus* Chub(*Leuciscus cephalus*	Field study Glatt River and tributaries (Switzerland)	[NP] = 3.9 ug/L in water	6 15 7	Ahel et al., 1993
NP	Bluegill sunfish *Lepomis macrochirus*	Field littoral enclosures Commercial NP applied every 2 days over 20 day period [4] dosages (3, 30, 100, 300 ug/L)	Maximum NP levels in the water column 2 h after application averaged 5 ± 4, 23 ± 11, 76 ± 21, and 243 ± 41 ug/L, respectively	87	Liber et al., 1999
NP	Ayu fish *Plecoglossus altivelis*	Field study Rivers flowing into Lake Biwa (Japan)	[NP] river = 0.14-3.08 ug/L [NP] fish = 10-110 mg/kg (wet weight)	21	Tsuda et al., 2001

Bioaccumulation Conclusions

Based on a comprehensive search of the literature, it is apparent that considerable information is readily available on the BAF and BCF potentials for NP in fish. Results are available for high quality studies conducted according to established guidelines, as well as research projects, which contribute to the overall weight of evidence. Valid laboratory studies conducted with both linear and branched NP isomers indicate that BCF values range from 90 to 741 L/kg wet weight, although the majority of the values fell in the range of 200 to 350 L/kg for the various fish species. BCF values for studies judged 'use with caution' ranged from 75 to 1300 L/kg wet weight. The higher BCF values are likely due to the fact that they were derived from total radioactivity measurements and do not account for the biotransformation to readily excretable metabolites which has indeed been demonstrated to occur. Further, the similarities in structure and log K_{ow} values as well as comparable BCF values for the various test chemicals support the conclusion that

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[87] Hu, J., Jin, F., Wan, Y., Yang, M., An, L. An, W., and Tao, S. (2005). Trophodynamic behavior of 4-nonylphenol and nonylphenol polyethoxylate in marine aquatic food web from Bohai Bay, North China: comparison to DDTs. Environ. Sci. Technol., 39, 4801-4807.

the uptake, partitioning, and metabolism behavior of linear and branched NP isomers are essentially identical in fish. .

Results of several field studies are also available for assessing the BAF for NP, including mesocosm studies where littoral enclosures were dosed with the test chemical. It is important to note that the results of field studies, where NP concentrations are measured in both the water column and in fish tissues, would be expected to be representative of the environmental behavior of the commercially relevant, branched isomers. BAF values calculated from field studies ranged from 6 to 87 L/kg wet weight. None of the BAF values exceed the proposed Washington State criteria for categorization as bioaccumulative. Further, the fact that field determined BAF values are lower than laboratory measured BCF values indicates that trophic magnification is not expected.

In summary, an examination of fish uptake and elimination constants indicate that all NP isomers demonstrate uptake into fish that is significantly attenuated by rapid elimination/metabolism processes in fish, resulting in generally low to moderate laboratory BCF values and no significant bioaccumulation in field studies. Overall, the weight of evidence supports the conclusion that NP does not meet the Washington State proposed criteria to be categorized as bioaccumulative.

V.   SUMMARY AND CONCLUSION

While it is acknowledged that NP is toxic to aquatic species and EPA has proposed a chronic Water Quality Criteria of 5.9 micrograms per liter as protective of the freshwater environment for this compound, numerous data available on the compound's persistence and bioaccumulative properties collectively support the conclusion that NP does not meet the proposed criteria for persistence and bioaccumulation in the Revised Draft Washington State PBT Rule. In addition, this conclusion is consistent with those in previous governmental assessments.[88,89,90,91,92]

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[88] Environment Canada and Health Canada. (2001). Priority Substances List Assessment Report: Nonylphenol and Its Ethoxylates.
[89] US EPA. (2004b). PBT Profiler. Prepared by Environmental Science Center for the Office of Pollution Prevention and Toxic Substances, US EPA. Version 1.203.
[90] Rodier, D. (1996). EPA RM-1 Document for Para-Nonylphenol.
[91] European Union. (2001). Risk Assessment Report: 4-Nonylphenol (branched) and Nonylphenol: Final Report. http://ecb.jrc.it/DOCUMENTS/Existing-Chemicals/RISK_ASSESSMENT/REPORT/
[92] European Chemicals Bureau. (2003). PBT Working Group Substance Information Sheets for Nonylphenol (CAS 25154-52-3) and Phenol, 4-Nonyl, branched (CAS 84852-15-3).